

WHAT IS CLAIMED IS:

1. A pig that lacks any expression of functional alpha1,3 galactosyltransferase.
- 5 2. An organ of a pig that lacks any expression of functional alpha1,3 galactosyltransferase.
3. The organ of claim 2, wherein the organ is a kidney.
- 10 4. The organ of claim 2, wherein the organ is a liver.
5. The organ of claim 2, wherein the organ is a heart.
6. The organ of claim 2, wherein the organ is a lung.
- 15 7. The organ of claim 2, wherein the organ is a pancreas.
8. A tissue of a pig that lacks any expression of functional alpha1,3 galactosyltransferase.
- 20 9. The tissue of claim 8, wherein the tissue is cartilage.
10. The tissue of claim 8, wherein the tissue is bone.
- 25 11. The tissue of claim 8, wherein the tissue is adipose.
12. The tissue of claim 8, wherein the tissue is muscle.
13. A cell or a cell line from a pig that lacks any expression of functional alpha1,3 galactosyltransferase.
- 30 14. The cell of claim 13, wherein the cell is derived from the pancreas.
15. The cell of claim 14, wherein the cell is an Islet of Langerhans cell.

16. The cell of claim 14, wherein the cell is an insulin secreting cell.
17. A method for the production of a pig that lacks any expression of functional alpha1,3 galactosyltransferase comprising: breeding a male pig heterozygous for the alpha-1,3-GT gene with a female pig heterozygous for the alpha-1,3-GT gene.
18. The method of claim 17, wherein one or both pigs are heterozygous due to the genetic modification of one allele of the alpha-1,3-GT gene to prevent expression of that allele.
19. The method of claim 17, wherein one or both pigs are heterozygous due to the presence of a point mutation in an allele of the alpha-1,3-GT gene.
20. The method of claim 19, wherein the point mutation is a T-to-G point mutation at the second base of exon 9 of the alpha-1,3-GT gene.
21. The method of claim 17, wherein a male pig that contains a T-to-G point mutation at the second base of exon 9 of the alpha-1,3-GT gene is bred with a female pig that contains a T-to-G point mutation at the second base of exon 9 of the alpha-1,3-GT gene.
22. A method for producing an alpha 1,3 GT deficient non-human animal comprising:
- (a) exposing a population of cells to *C. difficile* toxin A;
 - (b) removing cells which lift from the surface matrix because they are adversely affected by toxin A due to the receptor-mediated cytotoxicity of the toxin;
 - (c) expanding and maintaining those cells which do not show the effects of receptor-mediated cytotoxicity;
 - (d) using these toxin A-resistant cells as nuclear donors for nuclear transplantation into a suitable recipient cell;

- (e) implanting the fused and activated cells into a female surrogate;
and
- (f) producing a cloned animal which exhibits a deficiency or complete lack of gal alpha1,3-gal epitopes on its cell surfaces.

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23. The method of claim 22, wherein the cells to be selected for have been rendered heterozygous with respect to the gal alpha1,3 allele, via targeted knockout of one allele by homologous recombination.

10 24. The method of claim 22, wherein the cells to be selected for have been rendered homozygous with respect to the gal alpha1,3 allele, via targeted knockout of both alleles by homologous recombination.

25. The method of claim 22 wherein the cells to be selected for have been
15 rendered heterozygous with respect to the gal alpha1,3 allele via a natural mutation of a single gal alpha1,3 allele, which disables the alpha 1,3 galactosyltransferase gene.

26. The method of claim 22 wherein the cells to be selected carry an alpha
20 1,3 gal double knockout created by targeted knockout of one allele by homologous recombination and natural mutation of the second allele.

27. The method of claim 22 wherein the cells to be selected for are
homozygous with respect to the gal alpha1,3 allele accomplished via natural
25 mutations of both gal alpha1,3 alleles, which disables the alpha 1,3 galactosyltransferase gene.

28. The method of claim 22 wherein the cells to be selected for carry an
alpha 1,3 gal double knockout accomplished via targeted knockout of one
30 allele by homologous recombination and natural mutation of the second allele which disables the alpha 1,3 galactosyltransferase gene.

29. The method of claim 22, wherein the cells to be selected for have been rendered homozygous with respect to the gal alpha1,3 allele via induced mutations of both gal alpha1,3 alleles, which disables the alpha 1,3 galactosyltransferase gene.
- 5 30. The method of claim 22 wherein the *Clostridium difficile* toxin A used for selection is in the form of a purified toxin.
- 10 31. The method of claim 22 wherein the *Clostridium difficile* toxin A used for selection is in the form of a culture supernatant fluid.
32. The method of claim 22 wherein the purified toxin is applied to dispersed cells, and wherein said dispersed cells are then cultured *in vitro* in vessels suitable for cell adherence.
- 15 33. The method of claim 22, wherein the purified toxin is applied to adhered cells.
- 20 34. The method of claim 22, wherein the culture supernatant fluid is applied to dispersed and un-adhered cells followed by culturing in vessels suitable for cell adherence.
35. The method of claim 22, wherein the culture supernatant fluid is applied
- 25 36. The method of claim 22, wherein said animal is a pig.
37. The method of claim 22, wherein a mutation is induced by a mutagenic agent selected from the group consisting of a chemical mutagen, radiation, and a transposon.
- 30 38. The method of claim 22, wherein said chemical mutagen is selected from the group consisting of EMS, ENU, mustard gas and ICR191.

39. The method of claim 22, wherein said radiation is selected from the group consisting of ultraviolet radiation, alpha radiation, beta radiation and gamma radiation.
- 5 40. A cell that carries a homozygous knockout for the gal alpha-1,3-GT gene in which at least one allele contains a natural or spontaneous mutation in the gal alpha-1,3-GT gene, wherein said cell is produced by a method comprising:
- 10 (a) exposing a population of cells to *C. difficile* toxin A;
- (b) removing cells which are adversely affected by toxin A due to the receptor-mediated cytotoxicity of the toxin; and
- (c) expanding and maintaining a cell that does not show the effects of receptor-mediated cytotoxicity.
- 15 41. The cell of claim 40, wherein said cell carries a homozygous knockout for the gal alpha-1,3-GT gene in which at least one allele contains the base substitution thymine to guanine at base position 424 of the alpha 1,3 GT gene, resulting in the amino acid substitution tyrosine to aspartic acid at position 142 in the gal alpha-1,3-GT protein.
- 20 42. The cell of claim 40, wherein said cell carries a homozygous knockout for the gal alpha-1,3-GT gene in which at least one allele contains an induced mutation in the gal alpha-1,3-GT gene.
- 25 43. An animal produced according to the method of claim 22.
44. An animal produced by nuclear transfer cloning using the cell of claim 40 as a nuclear donor.
- 30 45. An animal produced by nuclear transfer cloning using the cell of claim 41 as a nuclear donor.

46. An animal produced by nuclear transfer cloning using the cell of claim 42 as a nuclear donor.

5 47. Cells, tissues, and organs obtained from the animal of claim 42 for use as an in vivo or ex vivo supplement or replacement for recipient cells, tissues or organs.

10 48. Cells, tissues, and organs obtained from the animal of claim 43 for use as an in vivo or ex vivo supplement or replacement for recipient cells, tissues, or organs.

15 49. Cells, tissues, and organs obtained from the animal of claim 44 for use as an in vivo or ex vivo supplement or replacement for recipient cells, tissues, or organs.

50. Cells, tissues, and organs obtained from the animal of claim 45 for use as an in vivo or ex vivo supplement or replacement for recipient cells, tissues, or organs.

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